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Carbon, Nitrogen, and Nutrient Interactions in *Beta vulgaris* L. as Influenced by Nitrogen Source, NO_3^- versus NH_4^+

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Sugar beets (*Beta vulgaris* L. cv F58–554H1) were grown hydroponically in a 16-h light, 8-h dark period (photosynthetic photon flux density of 0.5 mmol m^{-2} s^{-1}) for 4 weeks from sowing in half-strength Hoagland nutrient solution containing 7.5 mM nitrate. Half of the plants were then transferred to 7.5 mM ammonium N; the rest remained in solution with 7.5 mM nitrate N. Upon transfer from nitrate to ammonium, the total N concentration decreased sharply in the fibrous roots and petiole/midribs and increased substantially in the leaf blades. This was because of the decreased nitrate concentrations in fibrous roots and petioles and a concomitant increase in amino acid/amide-N and protein N in leaf blades. Sugar beets acclimated to ammonium partly by a 2.5-fold increase in glutamine synthase activity in fibrous roots and a 1.7-fold increase in leaf blades. Rapid ammonium assimilation into glutamine consumed carbon skeletons, leading to a depletion of foliar starch, sucrose, and maltose. Ammonium treatment stimulated activities of some glycolytic/Krebs cycle enzymes, e.g. pyruvate dehydrogenase. Nitrate-fed leaf blades contained substantially larger concentrations of osmolytes (i.e. nitrate, cations, and sucrose), which may have contributed to the faster rates of leaf expansion in nitrate-fed compared to ammonium-fed plants.

Sugar beet plants supplied with ammonium rather than with nitrate as an N source have reduced rates of growth that result from decreased rates of expansion of individual leaves and not from diminished rates of photosynthesis per unit leaf area (Raab and Terry, 1994). The diminished leaf expansion with ammonium treatment was a consequence of a decreased expansion of leaf cells. Also, the chloroplasts in ammonium-supplied plants appeared to be a major repository for the products of ammonium assimilation (Raab and Terry, 1994).

Ammonium ions entering the roots of plants are immediately assimilated by the GS-GOGAT system to form Gln and glutamate (Mäck and Tischner, 1990; Amancio and Santos, 1992), which requires a substantial contribution of fixed C in both roots and leaves. In roots, C is required directly as α-KG (metabolism of Suc through glycolysis/Krebs) and indirectly for the generation of reducing power (reduced Fd) in the plastids. In leaves, triose-P enters the glycolytic pathway directly. The consumption of fixed C during the assimilation of ammonium thus can appreciably reduce carbohydrate stores (Mehrer and Mohr, 1989).

The uptake and transport differs for ammonium N and nitrate N. Whereas ammonium ions are assimilated in roots and transported as amino acids and amides to the leaves, nitrate ions are transported to leaves largely as nitrate (Arnozis and Findenegg, 1986; Shelp, 1987). Thus, the form of N ion supplied to roots influences the absorption and transport of other nutrients, including cations K^+, Ca^{2+}, Mg^{2+}, and Na^+ and anions SO_4^{2-}, PO_4^{3-}, and Cl^- (Pilbeam and Kirkby, 1992).

In the present paper, we examine the interactions of N source, ammonium versus nitrate N, on the metabolism of C and N and on the uptake and transport of other nutrient ions. The perturbations of C metabolism after switching plants from nitrate N to ammonium N were followed over time as changes in nonstructural carbohydrates, i.e. starch, Suc, maltose and Glc, and as changes in the activities of certain enzymes in the glycolytic and Krebs cycle pathway and of PEPc. The alterations in N metabolism with N source were followed as changes in total N and its inorganic N components, in free amino acids and amides, and in the activities of GS in fibrous roots and leaves.

MATERIALS AND METHODS

Plant Culture

Sugar beets (*Beta vulgaris* L. cv F58–554H1; U.S. Department of Agriculture, Salinas, CA) were sown in vermiculite and grown for 2 weeks at 25°C and in growth chambers with a 16-h light period at a PPFD of 0.5 mmol m^{-2} s^{-1}. The seedlings were transplanted to continuously aerated hydroponic culture containing one-half-strength modified Hoagland solution (Terry, 1980) with nitrate as the sole N source at a density of 24 seedlings per 15-L tank for an additional 2 weeks of pretreatment. Treatments then were

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Abbreviations: ADPG, adenosine 5′-diphosphoglucose; α-KG, α-oxoglutarate; ATP-PFK, ATP:fructose-6-phosphate-1-phosphotransferase; DAT, days after transplanting; FBP, fru 1,6-bisphosphate; GABA, γ-aminobutyric acid; GOGAT, glutamate synthase; GS, glutamine synthase; HPI, hexose-phosphate isomerase; PDC, pyruvate dehydrogenase complex; PEPc, phospho*enol*pyruvate carboxylase; PGM, phosphoglucomutase; PK, pyruvate kinase; PPi-PFK, PPi:fructose-6-phosphate-1-phosphotransferase; SPS, sucrose-P synthase; UDPG, uridine 5′-diphosphoglucose.

initiated by transferring one-half of the seedlings at one plant per 20-L container (plastic lined) to a solution containing 3.75 mM $Ca(NO_3)_2$ as the sole N source and the other seedlings were transferred to a solution containing 3.75 mM $(NH_4)_2SO_4$. Concentrations of all other nutrients remained as before except that 2.5 mM $CaCl_2$ was added to the ammonium solution. The culture solutions were buffered with solid $CaCO_3$ to maintain the pH at 6.7 (monitored every 2 d), and 1 M aqueous NaOH or HCl was added as necessary. Without such control, the pH of ammonium solutions would decrease to pH 4 in 6 to 7 d. Nutrient solutions were replaced every 4 d. No nitrate was detected in the ammonium nutrient solution, and nitrate contained in the plant tissues upon transfer to the ammonium solution disappeared within 3 d. The plants were grown for 3 weeks after treatments were initiated and harvested at 3-d intervals.

Leaf Blade Carbohydrates

Four hours into the light period on each harvest day, two leaf discs (3.88 cm^2/disc) from a rapidly expanding leaf (1.5–2 dm^2 area) were sampled from each of three plants of each treatment. The samples were frozen in liquid N and vigorously ground in a prechilled ceramic mortar and pestle. The leaf powder was then extracted with 80% (w/v) ethanol, and leaf blade Glc, Suc, and starch were determined as described by Fredeen et al. (1989) except that Boehringer Mannheim amyloglucosidase (catalog No. 1202 332) was utilized for starch digestion. Leaf blade maltose was determined in the clarified aqueous extract (delipidated with chloroform) used for Suc and Gln determinations by incubating it at a 1:1 volume ratio with 2000 units/L maltase (α-glucosidase from green *Coffea arabica* in 50 mM Mes-NaOH, pH 6.80) for 30 min. Glc release was determined as before and corrected for endogenous Glc. All leaf blade carbohydrates were determined as Glc and expressed on the basis of mmol C m^{-2} leaf area.

Foliar Enzymes of Starch and Suc Metabolism

At 12 DAT to either nitrate or ammonium, activities of ADPG pyrophosphorylase (EC 2.7.7.27), starch phosphorylase (EC 2.4.1.1), maltose phosphorylase (EC 2.4.1.8), β-amylase (EC 3.2.1.2), UDPG pyrophosphorylase (EC 2.7.7.9), cytosolic FBPase (EC 3.1.3.11), acid invertase (EC 3.2.1.26), and Suc synthase (EC 2.4.1.13) were extracted and assayed as described previously (Rao et al., 1990) except that the protease inhibitor ϵ-aminocaproic acid was included in the extraction media at 1 mM to enhance stability and all extracts were desalted in a 4°C cold room on Sephadex G-25 M columns prior to assay. The V_{max} assay for foliar SPS (EC 2.4.1.14) was performed according to the method of Walker and Huber (1989). Activity of starch synthase (EC 2.4.1.21) was assayed according to the method of Brown and Huber (1988) at 1 mM ADPG, which was similar to the concentration used above for ADPG pyrophosphorylase.

Foliar Enzymes of Glycolysis and Organic Acid Metabolism

Glucokinase (EC 2.7.1.1), HPI (EC 5.3.1.9), and PGM (EC 2.7.5.1) were assayed by the method of Rao et al. (1990). PK (EC 2.7.1.40) was assayed by the method of Baysdorfer and Bassham (1984). ATP-PFK (EC 2.7.1.11) and PPi-PFK (EC 2.7.1.90) were assayed by the methods of Doehlert (1990). PEPc (EC 4.1.1.31) was assayed by the coupled-enzyme method of Meyer et al. (1988) and corrected for apparent PEP phosphatase activity. Citrate synthase (EC 4.1.3.7) was assayed by the 5,5′-dithiobis(2-nitrobenzoic acid)-coupling reaction described by Bajracharya and Schopfer (1979). Overall PDC activity was assayed by the coupled-enzyme method of Taylor et al. (1992) as acetyl CoA-dependent NADH reduction. All extracts were desalted on Sephadex G-25 M in a 4°C cold room prior to assay.

Leaf Blade and Fibrous Root GS

GS (EC 6.3.1.2) was assayed by the coupled-enzyme method of Stewart and Rhodes (1977).

Total C, N, and C-Stable Isotope Composition

Dried leaf blade, petiole, fibrous root, and storage root tissues from three plants of both N sources were ground to pass a 60-mesh screen on a Wiley mill and reground to flour consistency in a ball mill, and 5- to 7-mg subsamples were weighed on a Cahn C-30 microbalance into tin combustion cups (Conroy Scientific, Costa Mesa, CA). Total C and N and their respective isotope abundances were determined on a Europa Scientific (Crewe, UK) Tracermass Stable Isotope analyzer, following combustion in a Roboprep-CN Biological Sample Converter. National Bureau of Standards (Gaithersburg, MD) NBS-1572 citrus leaves were analyzed after every 10 samples as an internal standard to correct for drift of the mass spectrometer. The δ ^{13}C ratios are expressed by the per mil deviation from the value for Pee Dee Belemnite using a secondary standard according to the method of Ehleringer (1988).

Inorganic N Speciation

Free ammonium and [nitrate plus nitrite] were extracted overnight from oven-dried (55°C) leaf, petiole, and fibrous root material of three plants of each N source in 2% (w/v) acetic acid in a reciprocating shaker at 35°C. The extracts were centrifuged and the supernatants quantified the following day using colorimetric methods on a Lachat Autoanalyzer (Lachat Instruments, Milwaukee, WI, 1990). Briefly, [nitrate plus nitrite] was converted to nitrite after passage over a Cu/Cd reduction column and analyzed by a diazotization reaction with sulfanilamide followed by coupling to *N*-(1-naphthyl)ethylenediamine dihydrochloride. Ammonium was quantified by the salicylate/hypochlorite chemistry. Values are expressed on a dry weight basis for direct comparison with total N determinations as described above.

Plant Tissue Analyses for Na^+, K^+, Ca^{2+}, and Mg^{2+}

Subsamples (100 mg) from the dried plant material used for C and N analyses were pre-extracted overnight with 3 mL of concentrated HNO_3, treated the next morning with 2:1 (v/v) $HNO_3/HClO_4$, and heated. The clarified solutions were made up to a 50-mL volume with double-distilled water and then quantified for major cations on a Perkin-Elmer Plasma 40 spectrometer using inductively coupled plasma/atomic emission spectroscopy at wavelengths of 590 nm (Na^+), 766.49 nm (K^+), 317.93 nm (Ca^{2+}), and 279.09 nm (Mg^{2+}).

Total S and Sulfate-S

Subsamples (100 mg) from the dried plant material at 21 DAT used for C and N analyses were pre-extracted overnight with 3 mL of concentrated HNO_3, treated with 1:1 (v/v) $HNO_3/HClO_4$, and heated. The clarified solutions were made up to a 25-mL volume and measured for total S using inductively coupled plasma/atomic emission spectroscopy at a wavelength of 180.731 nm on a Perkin-Elmer Plasma 40 spectrometer, the optics of which had been purged the preceding 12 h with N_2 gas. Sulfate-S was extracted with 2% (w/v) acetic acid and separated from organic S using the column-chromatographic method of Littlefield et al. (1990), and the resulting S was determined as before by inductively coupled plasma spectroscopy. Organic S was calculated by subtracting sulfate from total S.

P Fractionation

Total P ($HClO_4$ extracted) and 2% (w/v) acetic acid-soluble P were estimated for both leaves and fibrous roots at 12 DAT according to the method of Fredeen et al. (1989) on a Perkin Elmer Plasma 40 inductively coupled plasma spectrometer at 213 nm. Organic P (mainly esterified phosphates) was estimated by subtracting 2% acetic acid-soluble P from total P.

Amino Acid Pools

Three fibrous root samples (1–1.2 g fresh weight) and three 3.88-cm^2 sugar beet leaf discs pooled from three replicate plants of each N source were harvested 4 h into the light period and vigorously ground in liquid N_2. Tissues were then extracted with 10 mL of 80% (v/v) ethanol on a reciprocating shaker for 2 h at 30°C. The resulting brei was centrifuged for 20 min at 20,000*g*. The supernatants were decanted and dried at 35°C with filtered laboratory air. Amino acid analysis was performed at the Protein Structure Laboratory, University of California, Davis, on a Beckman 6300 amino acid analyzer, using α-amino-β-guanidino-propionic acid as the internal standard. Cys, Met, and Trp were not quantified.

Chemicals

All chemicals and biochemical reagents were of analytical grade (or higher) and were purchased from Sigma with the exception of amyloglucosidase (Boehringer-Mannheim), ε-aminocaproic acid (Calbiochem), and Sephadex G-25 M desalting columns (Pharmacia).

RESULTS AND DISCUSSION

The Fate of Ammonium or Nitrate Ions Entering Roots

When sugar beet plants were transferred from nitrate to ammonium, the total N concentration of the fibrous roots decreased by 27% within 3 d and then remained fairly constant with time (Fig. 1a). The initial decline was associated with the disappearance of nitrate (Fig. 1b), which

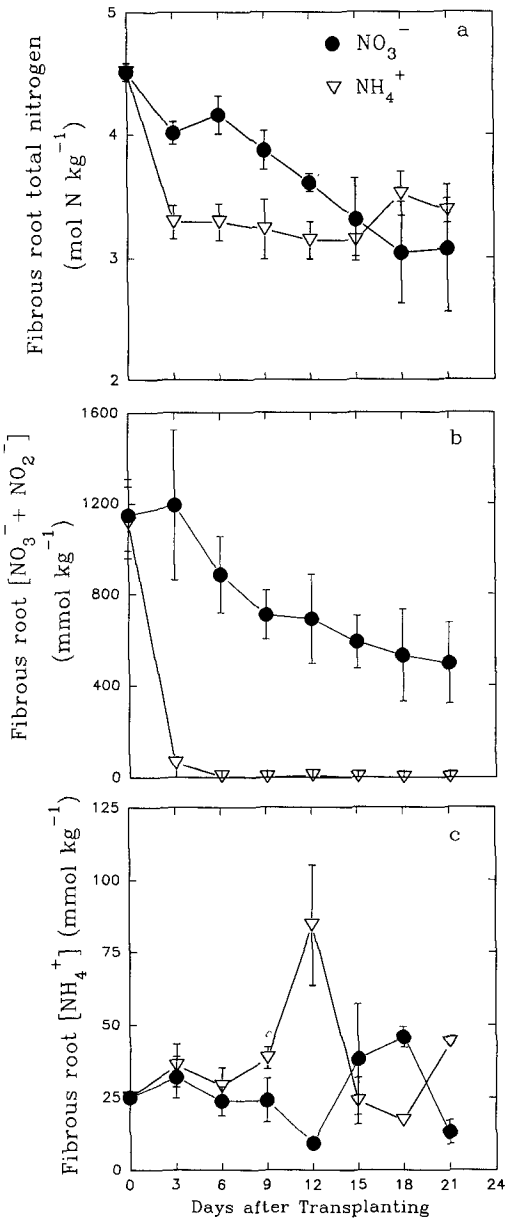

Figure 1. Time courses for the concentrations (dry weight basis) of total N (A), nitrate plus nitrite (B), and ammonium (C) of fibrous roots of sugar beets supplied with either nitrate N (●) or ammonium N (△). Values are means ± SD of four replicate plants for each treatment.

Table I. *Free amino acid pools from fibrous roots and leaf blades of sugar beet plants grown 12 DAT on either nitrate or ammonium as the N source*

Values are expressed on tissue dry weight basis. Met, Cys, and Trp were not assayed.

Amino Acid	Fibrous Roots		Leaf	
	Nitrate	Ammonium	Nitrate	Ammonium
	$\mu mol\ kg^{-1}$		$mmol\ kg^{-1}$	
Ala	51.5	154	1.85	2.17
Arg	ND[a]	ND	ND	30.8
Asn	103	193	ND	3.03
Asp	238	735	6.36	2.94
GABA	146	435	ND	1.29
Gln	72.8	280	6.60	69.0
Glu	121	251	13.8	17.9
Gly	28.0	137	0.676	0.890
His	ND	ND	ND	4.82
Ile	4.8	16.1	0.415	1.08
Leu	11.8	27.5	0.256	1.57
Lys	ND	27.8	ND	0.740
Phe	4.9	12.8	0.338	0.433
Pro	9.1	34.1	0.362	0.943
Ser	79.8	350	1.70	8.88
Thr	23.7	96.8	0.636	1.91
Tyr	ND	ND	0.654	3.53
Val	8.3	38.2	0.681	2.71

[a] ND, Not detected.

represented 25% of N in fibrous roots at 0 DAT. In the plants supplied with nitrate, the N concentration in fibrous roots decreased more slowly with time and reached similar concentrations to those of ammonium roots within 2 weeks. Conversely, total N concentration of storage roots did not change with time or treatment (data not shown). The concentration of ammonium ions did not change significantly in fibrous roots with time or treatment (Fig. 1c).

Concentrations of free amino acids in the fibrous roots of ammonium plants were 2-to 4-fold greater than those for nitrate-supplied plants (Table I). The five most significant amino acids (quantitatively in descending order) under ammonium nutrition were Asp, GABA, Ser, Gln, and Glu and under nitrate nutrition they were aspartate, GABA, glutamate, Asn, and Ser. With the exception of Ser, the dominant free amino acids in both nitrate- and ammonium-treated plants appeared to be the immediate products of glutamate metabolism. Ser most likely was formed from glycolysis via phosphoglycerate and hydroxypyruvate and not from glutamate.

If, as is generally believed (see reviews by Salsac et al., 1987; Pilbeam and Kirkby, 1992), ammonium ions are toxic, plants can acclimate to ammonium in the environment by increasing their enzymatic capacity in roots to assimilate ammonium via the GS-GOGAT pathway. By 12 DAT, exposure to ammonium increased the total extractable activity of GS in fibrous roots 2.5-fold from 32.3 ± 9.48 $\mu mol\ g^{-1}$ fresh weight h^{-1} for nitrate-fed plants to 80.5 ± 4.93 $\mu mol\ g^{-1}$ fresh weight h^{-1} for ammonium-fed plants (significant at the $P < 0.05$ level by Student's t test). An even larger increase was reported by Mäck and Tischner (1990). The increases in concentration that we observed in the immediate products of the GS-GOGAT cycle (Gln, Glu, GABA, and Asp; Table I) also suggest that the capacity of the GS-GOGAT enzyme system increased under ammonium nutrition. The concentrations of GABA and Arg in xylem are increased by ammonium nutrition (Hocking, 1980; Miflin and Lea, 1980). The high levels of these glutamate derivatives in the xylem suggests that the flow of both C

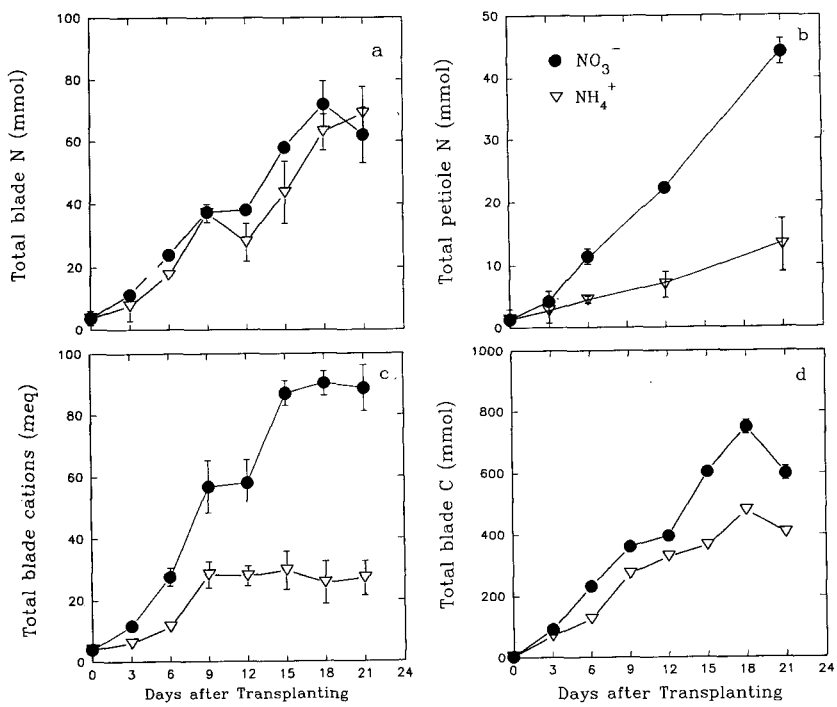

Figure 2. Time courses for the accumulation of total N by leaf blades (a), total N accumulation by petioles (b), cations ($K^+ + Na^+ + Ca^{2+} + Mg^{2+}$) accumulated by leaf blades (c), and total C accumulation by leaf blades (d). Values are means ± SD for four replicate plants of each treatment. Symbols are as in b.

and N through glutamate was increased in ammonium-supplied roots.

The Fate of Ammonium- or Nitrate-Derived N Entering Leaves

Even though plants grew more slowly under ammonium than nitrate nutrition (Raab and Terry, 1994), their leaf blades accumulated N at nearly equal rates (Fig. 2a). The total N concentration in the blades of ammonium plants thus had increased by 25% at 6 DAT and remained at high levels for the remainder of the growth period (Fig. 3a). In nitrate-supplied plants, however, total N concentrations of the leaf blades decreased with time.

In contrast to the leaf blades, ammonium nutrition resulted in a dramatic decrease in the N concentration of the petiole; under nitrate nutrition total N of petioles remained high during most of the growth period (Fig. 3b). The N in petioles of nitrate-fed plants consisted primarily of [nitrate plus nitrite], which at 3 DAT constituted 94% of the total N in the petioles and at 21 DAT constituted about 63% (Fig. 3d). The [nitrate plus nitrite] in leaf blades was much lower than in petioles. In ammonium-fed plants very little nitrate remained in either blades or petioles by 3 DAT (Fig. 3, c and d). Much of the nitrate in leaf blades and petioles apparently is stored in the vacuoles (Martinoia et al., 1981; Granstedt and Huffaker, 1982).

Ammonium ion concentrations in blades and petioles of nitrate-fed plants were fairly constant with time (Fig. 3, e and f). For plants transferred to ammonium, ammonium ion concentrations in the petiole increased 2.3-fold within the first 3 d before decreasing to concentrations characteristic of nitrate-fed plants. This transient increase in petiole ammonium indicates that assimilation of ammonium in roots initially was less than uptake but the roots rapidly adapted. Alternatively, rates of assimilation in roots did not adapt but the capacity for transport to the blades increased. A slight increase in the concentrations of ammonium ions occurred in leaf blades of ammonium-fed plants during the first 3 d and were sporadically higher than in nitrate-fed leaves after 9 d.

Ammonium nutrition increased the concentration of free amino acids in the leaf blades substantially (Table I). Concentrations of total α-amino N, which was 34.3 mmol kg^{-1} for nitrate-fed plants, were increased 4.5-fold in ammoni-

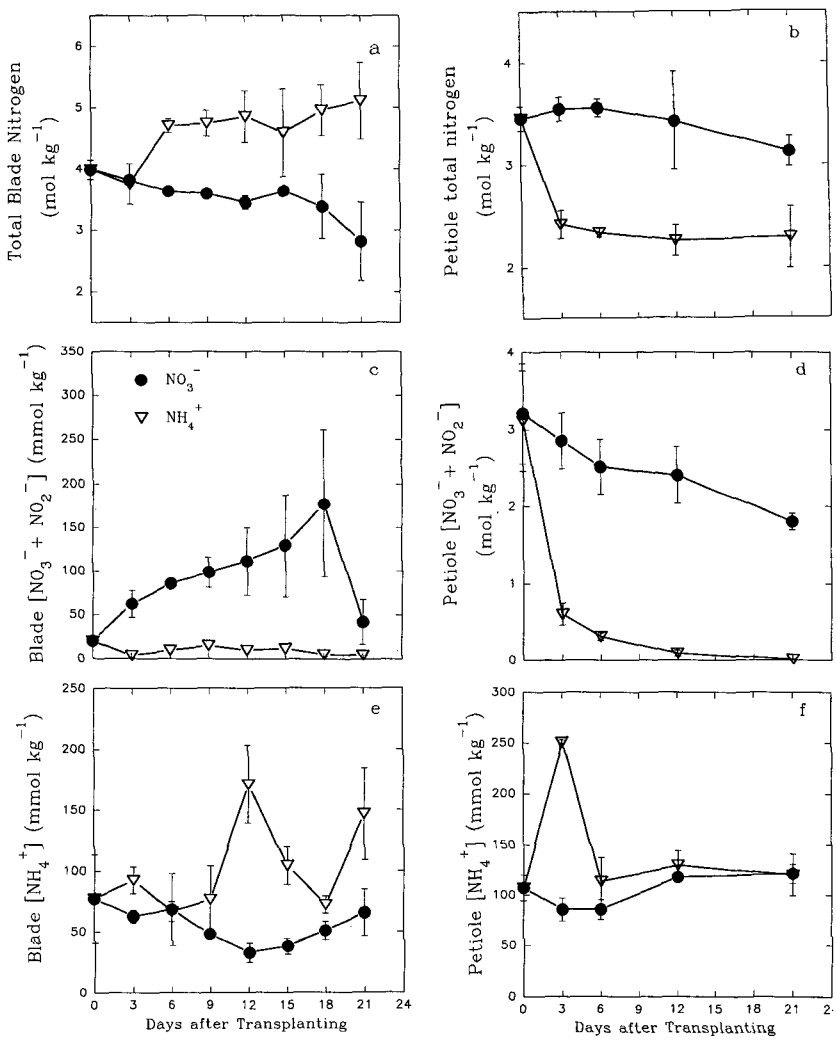

Figure 3. Time courses for the concentrations (dry weight basis) of leaf blade total N (a), petiole total N (b), leaf blade nitrate plus nitrite (c), petiole nitrate plus nitrite (d), leaf blade ammonium ions (e), and petiole ammonium ion (f) for sugar beet plants grown with either nitrate N or ammonium N. Values are means ± SD for four replicate plants of each treatment. Symbols are as in c.

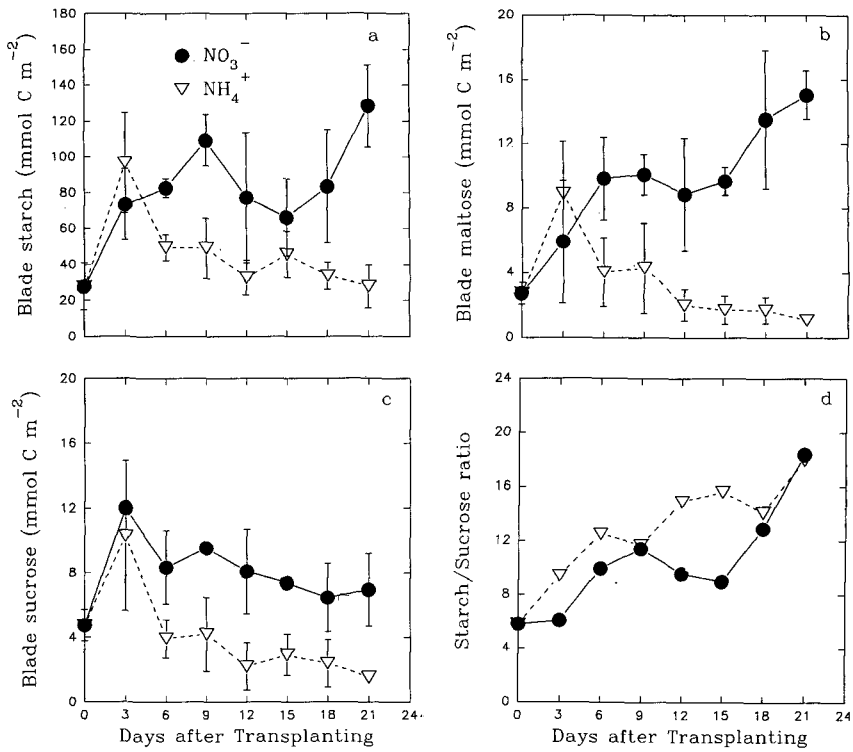

Figure 4. Time courses for nonstructural carbohydrates (expressed as mmol C m^{-2} leaf area) in the leaf blades: starch (a), maltose (b), Suc (c), and starch/Suc ratio (d). Values are means ± SD for three replicate plants of each treatment. Symbols are as in a.

um-grown leaves to 155 mmol kg^{-1}. Similarly, the 246 mmol kg^{-1} of N in free amino acids in leaves of ammonium-fed plants was 5.4-fold larger than the 45.6 mmol kg^{-1} in leaves of nitrate-fed plants. Of the total N concentration in the leaf blades of ammonium-fed plants, 5.1% was accounted for as amino acid/amide N, 2.7% was accounted for as ammonium N, and 0.2% was accounted for as nitrate (plus nitrite). Of the total N in nitrate plants, amino acid/amide N constituted 1.0%, nitrate (plus nitrite) constituted 3.2%, and ammonium N constituted 0.7%. The residual N for plants of both N sources resides in protein/nucleic acid N; under ammonium nutrition we previously observed a 4.3-fold increase in leaf soluble protein (Raab and Terry, 1994).

Ammonium nutrition also changed the composition of free amino acids in leaves (Table I). On a dry weight basis, Gln was 10.5-fold higher and Ser was 5.2-fold higher in ammonium-supplied leaf blades than in nitrate-supplied leaf blades. Gln, which constituted about 56% of the total amino acid/amide N pool in the leaf blades of ammonium-fed plants at 12 DAT and is found in both the xylem and phloem (Shelp, 1987), is important in both transport and assimilation of ammonium-derived N. Leaf blades of ammonium-fed plants also accumulated Arg, which is derived

Table II. *Activities of leaf blade enzymes of starch and Suc metabolism in sugar beet plants grown with either nitrate or ammonium as sole N source for 12 DAT*

Values are means ± SD, n = 4 determinations, expressed on a leaf dry weight basis.

Enzyme	Leaf		Ammonium as Percent of Nitrate
	Nitrate	Ammonium	
	μmol kg^{-1} s^{-1}		
ADPG pyrophosphorylase	281 ± 86	406 ± 36	144
Starch synthase	14.8 ± 3.72	21.3 ± 1.87	144
β-Amylase	24.4 ± 6.65	7.63 ± 2.50[a]	31
Maltose phosphorylase	26.4 ± 5.70	22.7 ± 5.40	86
Starch phosphorylase	6.89 ± 1.54	5.91 ± 2.10	86
SPS (V_{max})	54.5 ± 14.4	35.4 ± 8.71	65
UDPG pyrophosphorylase	1510 ± 140	2070 ± 220[a]	137
Cytosolic FBPase	63.2 ± 24.2	85.4 ± 28.9	135
Suc synthase	51.9 ± 15.8	22.5 ± 6.42	43
Acid invertase	32.7 ± 15.4	64.2 ± 17	196

[a] Means differ significantly by Student's t test at $P < 0.05$ level.

Table III. *Activities of leaf blade enzymes of glycolysis and the TCA cycle in sugar beet plants grown 12 DAT to either nitrate or ammonium*

Values are means ± SD, $n = 4$ determinations, expressed on a leaf dry weight basis.

Enzyme	Nitrate	Ammonium
	μmol kg⁻¹ s⁻¹	
Hexokinase	39.3 ± 13.4	23.4 ± 7.45
PGM	479 ± 111	737 ± 153
HPI	309 ± 56.6	443 ± 95.3
ATP-PFK	74.6 ± 25.7	100 ± 30.2
PPi-PFK	48.2 ± 17.8	98.2 ± 12.1[a]
PK	84.4 ± 30.9	107 ± 22.8
PDC	2.13 ± 0.79	4.72 ± 1.45[a]
Citrate synthase	95.8 ± 14.7	73.6 ± 12.6
PEPc	609 ± 92.0	645 ± 354

[a] Means differ significantly by Student's *t* test at $P < 0.05$ level.

from metabolism of chloroplastic glutamate (Taylor and Stewart, 1981).

When N was supplied as ammonium, compared to nitrate, foliar GS increased 70% from 833 ± 154 to 1420 ± 143 μmol kg^{-1} dry weight s^{-1}. The bulk of the leaf blade activity assayed by our method arises from the chloroplast-specific isoform GS_2 (Wallsgrove et al., 1983). The increased activities of GS may have been a response to transport of ammonium ions from roots that is indicated by the "spikes" in ammonium ion concentration in petioles at 3 DAT and leaf blades at 12 and 21 DAT (Fig. 3, e and f) of ammonium-fed plants.

It is clear that large amounts of total N are accumulated specifically in the leaf blades when N is supplied as ammonium and that lesser amounts accumulate in petioles and fibrous roots (Figs. 1–3). Evans (1989) concluded that 75% of total N in leaves of nitrate-fed plants is in the chloroplasts. Since ammonium nutrition doubled the size of chloroplasts and much of the increase in leaf blade N in ammonium-fed plants is attributable to N accumulation in chloroplast protein (Raab and Terry, 1994), it appears that most of the large flux of N to leaves in ammonium-supplied plants accumulates in the chloroplasts, mainly as stromal rather than thylakoid protein (Raab and Terry, 1994).

Consumption of C Skeletons for Gln Assimilation and Its Impact on Foliar Carbohydrate Levels

In plants cultured on ammonium, the ammonium is converted in roots to amino acids and amides, particularly Gln, which are transported to the leaf. In the leaf Gln enters the chloroplast via a "Gln transporter" (Somerville and Ogren, 1983; Yu and Woo, 1988) and is converted to glutamate by GOGAT. The assimilation of Gln requires a substantial consumption of fixed C in the form of α-KG (GOGAT reaction) which is obtained from photosynthetically fixed triose-P entering the glycolytic and Krebs cycle pathways. In fact, the availability of α-KG may be a limiting factor in N assimilation as indicated by the studies of Magalhaes et al. (1992) and Monselise and Kost (1993).

The data indicate that the influx of N into the leaf blades of ammonium-supplied plants was not accompanied by a matching accumulation of photosynthetic C. Linear regression analysis of total N content during the 21-d growth period (Fig. 2a) showed that rates of N accumulation in blades were approximately 3.2 mmol N d^{-1} whether N was supplied as ammonium or nitrate. The rate of accumulation of C by leaf blades was substantially less for ammonium-fed plants than for nitrate-fed plants: nitrate-fed plants accumulated 34.2 mmol C d^{-1}, whereas ammonium-fed plants accumulated only 22.2 mmol C d^{-1} (Fig. 2d). Thus, the depletion of the nonstructural foliar carbohydrates that we observed in ammonium-fed plants (Fig. 4, a–c) could be due to the consumption of C skeletons necessary to assimilate the large influx of Gln into the leaf blades.

In nitrate-fed leaves, starch and maltose levels in the blades increased during the 21-d growth period (Fig. 4, a and b) during which time blade nitrate levels also increased (Fig. 3c). One might conclude that an insufficient capacity for nitrate reduction resulted in a diminished requirement for C skeletons for ammonium assimilation and led to a buildup in carbohydrates. Blade Glc levels (data not shown), which were variable over time and did not differ significantly between treatments, averaged 0.78 mmol C m^{-2} for nitrate-supplied plants and 0.63 mmol C m^{-2} for ammonium-supplied plants. Suc levels also remained relatively constant in nitrate-fed leaves (Fig. 4c). The ratio of starch/Suc increased during the 21-day growth period in plants receiving both treatments (Fig. 4d).

The reduction in starch level in ammonium leaves was not associated with a reduction in total extractable activity of the ADPG pyrophosphorylase and starch synthetase, which are enzymes involved in starch synthesis (Table II). The in vivo activity of ADPG pyrophosphorylase in ammonium plants, however, may have been down-regulated by a lowering of the [3-phosphoglyceraldehyde]/[Pi] ratio (Heldt et al., 1977) since blade Pi concentration increased markedly. Of the starch-degrading enzymes measured, only α-amylase (significant at $P < 0.05$) was significantly decreased under ammonium nutrition relative to nitrate-supplied plants. Among the enzymes involved in the synthesis of Suc, ammonium decreased the extractable activity of SPS, had little effect on cytosolic FBPase, and increased UDPG pyrophosphorylase activity (significant at $P < 0.05$) (Table II). With respect to Suc breakdown, nitrate-fed plants possessed much higher maximal activities of Suc synthase, whereas for ammonium-fed plants the activity of acid invertase doubled (Table II).

Table IV. *Cation concentrations of fibrous roots of sugar beets grown with either nitrate or ammonium*

Values are means ± SD, averaged over all harvests 0 to 21 DAT, $n = 21$ determinations.

Cation	Nitrate	Ammonium
	mol kg⁻¹ dry wt	
K^+	1.66 ± 0.185	1.44 ± 0.215
Na^+	0.095 ± 0.029	0.085 ± 0.029
Mg^{2+}	0.267 ± 0.044	0.278 ± 0.046
Ca^{2+}	0.953 ± 0.196	0.879 ± 0.169

Glycolysis interacts with ammonium assimilation through the production of intermediates such as PEP, oxaloacetic acid, and pyruvate, all of which may serve as precursors to their respective large families of amino acids. In particular, triose-P from the chloroplast produces pyruvate (glycolysis) that enters the mitochondria to provide the acetyl CoA required for the Krebs cycle to regenerate α-KG utilized in the GOGAT reaction. Ammonium treatment increased the total extractable activities of some enzymes in the glycolysis/Krebs cycle pathway, i.e. PPi-PFK and PDC activities (the increase in PDC, which is believed to be an important rate-controlling enzyme [Camp and Randall, 1985], was more than 2-fold, Table III). On an area basis, the activities of PGM, HPI, and PPi-PFK were increased by 78, 66, and 136%, respectively (significant at $P < 0.05$ except for PPi-PFK, $P < 0.01$). Interaction of ammonium assimilation with glycolysis was also observed by Geigenberger and Stitt (1991), who showed that supplying *Ricinus* seedlings with either Gln or NH_4Cl increased the activities of glycolytic enzymes and doubled the rate of glycolysis.

Algae, in contrast to higher plants, can replenish C intermediates used in ammonium assimilation by the action of PEPc, which produces oxaloacetic acid for the Krebs cycle (Guy et al., 1989; Turpin et al., 1991; Coronil et al., 1993). In sugar beet plants, the form of N supplied had little effect on total extractable activity of leaf blade PEPc (Table III). Furthermore, C-stable isotope $\delta^{13}C$ discrimination in whole leaf blades of ammonium-fed plants indicated that only a tiny portion of the C fixed by ammonium-fed plants was by PEPc compared to Rubisco. When the PEPc activities were obtained at 12 DAT, $\delta\ ^{13}C$ were -28.6 ± 0.6 for nitrate-supplied plants and -29.1 ± 0.5 per mil for ammonium-supplied plants. Our results are consistent with those of Melzer and O'Leary (1987), who showed that less than 5% of the total C fixed in tobacco was supplied by PEPc, and with Winter et al. (1982), who found that ammonium nutrition decreases the contribution of PEPc relative to Rubisco in the C_3/C_4 intermediate, *Moricandia arvensis*.

Interaction of Ammonium and Nitrate Nutrition with the Uptake and Transport of Other Ions

N source did not greatly affect the concentrations of K^+, Ca^{2+}, Na^+, and Mg^{2+} in the fibrous roots (Table IV) but had a marked effect on these cations in the leaf blades (Fig. 5). In ammonium-fed plants N is moved as Gln (plus other amino acids and amides) in the xylem to the shoot, whereas in nitrate-fed plants N is moved primarily as nitrate ions (Arnozis and Findenegg, 1986; Shelp, 1987; van Beusichem et al., 1988). Nitrate moving in the xylem requires counterbalancing cations, the most important of which is K^+. Nitrate-treated leaf blades have higher concentrations of K^+, Ca^{2+}, Mg^{2+}, and Na^+ than ammonium-treated leaves (Fig. 5). With respect to the individual cations in the leaf blades, ammonium decreased Mg^{2+} and Ca^{2+} to a greater extent than either K^+ or Na^+.

The rate of accumulation of N by the blades of nitrate-fed plants (3.23 mmol N d^{-1}) was accompanied by a higher net rate of accumulation of cation charge equivalents (4.62 meq d^{-1}) (Fig. 2c); whereas in ammonium-fed plants, net cation accumulation was only 1.24 meq d^{-1} (N accumulation was 3.19 mmol N d^{-1}). The higher rate of cation accumulation in nitrate- versus ammonium-supplied plants was probably partly associated with the need for counterbalancing cations for nitrate moved in the xylem sap but may also

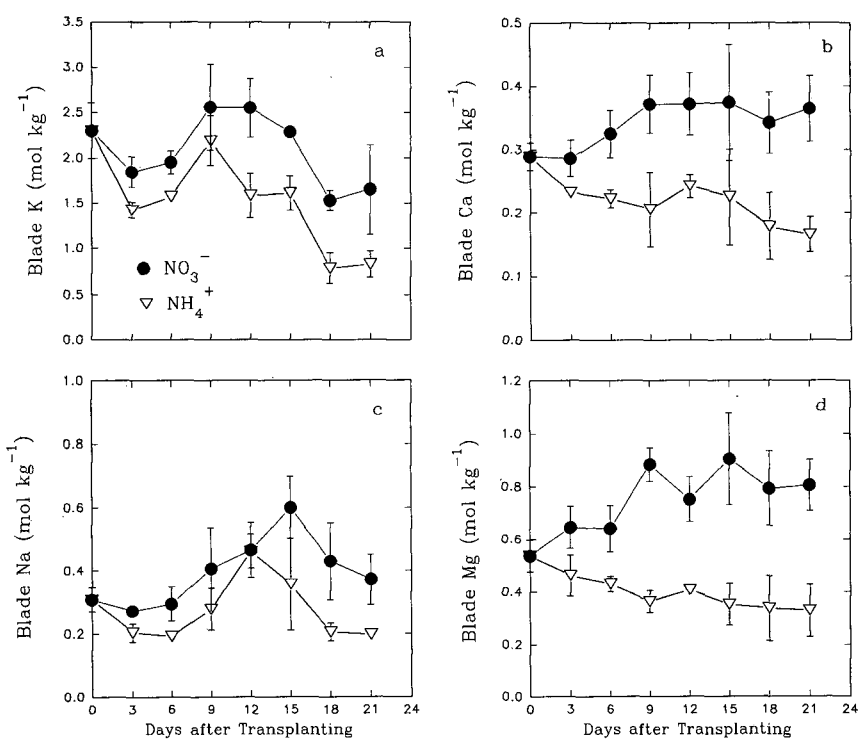

Figure 5. Time course for the concentrations of cations (dry weight basis) in leaf blades: K^+ (a), Ca^{2+} (b), Na^+ (c), and Mg^{2+} (d) for plants grown with either nitrate N or ammonium N. Values are means ± SD for three replicate plants of each treatment. Symbols are as in a.

Table V. *P and S partitioning within leaf blades and fibrous roots of sugar beet plants grown with either nitrate or ammonium as N source*

Values are means ± SD, $n = 4$ determinations; plants were sampled 12 DAT for P and 21 DAT for S.

Treatment	Total P	Pi	Organic P	Total S	Sulfate	Organic S
		mmol P kg^{-1} dry wt			*mmol S kg^{-1} dry wt*	
Nitrate blades	280 ± 25.2	17.1 ± 1.68	263	163 ± 12.8	10.4 ± 1.93[a]	153
Ammonium blades	988 ± 146[a]	57.4 ± 6.32[b]	931	180 ± 4.99[c]	4.60 ± 2.19	175
Nitrate roots	305 ± 22.6	12.9 ± 1.58	292	126 ± 10.9	1.96 ± 0.313	124
Ammonium roots	491 ± 45.5[a]	23.2 ± 1.79[a]	468	183 ± 11.2	5.94 ± 3.23	177

[a] Means differ significantly by Student's *t* test at $P < 0.01$. [b] Means differ significantly by Student's *t* test at $P < 0.001$. [c] Means differ significantly by Student's *t* test at $P < 0.05$.

have been associated with increased concentrations of organic anions (e.g. malate; Salsac et al., 1987).

The buildup of nitrate ions, cations, Suc (Fig. 4c), and possibly organic acids in leaf blades supports the view of Raab and Terry (1994) that nitrate-supplied plants have greater rates of leaf area expansion than ammonium-supplied plants because of increased levels of osmolytes necessary for cell expansion. This view extends that of Salsac et al. (1987) who suggested that the detrimental effect of ammonium nutrition on plants relates to the inability of plant cells to accumulate osmolytes necessary for osmotic adjustment under varying soil water potentials.

Although ammonium treatment had little effect on the concentrations of cations in fibrous roots, it substantially increased the uptake of some inorganic anions. Pi and organic P were substantially higher in both fibrous roots and leaf blades of ammonium-grown compared to nitrate-grown plants (Table V). Sulfate and total S concentrations in ammonium-grown plants were higher (although not significantly) in fibrous roots, and total S was higher in the blades (Table V). We did not obtain chloride values for the plant material of this experiment but, in a comparable study, chloride concentrations of leaf blades averaged over 14, 17, and 21 DAT were 1040 ± 191 mmol kg^{-1} for ammonium-grown plants compared to 323 ± 80.2 for nitrate-grown plants. Similar counterbalancing by Cl^- anions in ammonium-supplied sugar beets was observed by Findenegg et al. (1989).

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